used to test this assumption and refine parameters in the model that are otherwise difficult to measure directly, such as the volume occupied by PEG inside the pore and the local concentration of electrolyte, found to be approximately half the bulk value. MD simulations are also used to test a central hypothesis in the theoretical model that PEG complexes cations to acquire a net positive charge. We confirm that this is indeed the case and that five PEG subunits participate in forming crown-ether like sub-structures with a single cation. The refined theoretical model is then fit to blockade depth and residence time values measured experimentally as a function of PEG size (varying from 1000 g/mol to 2000 g/mol). We find that the theoretical predictions of the model agree quantitatively with experiment thereby validating its assumptions.

2679-Pos Board B698

Integration of Biogenic Nanopore Membranes on Prefabricated Fluidic Support Substrates

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Marine diatoms provide an alternative to machined silica nanopores, avoiding costly and slow throughput fabrication steps, while being able to achieve pore structures with diameters on the order of 40 nm. The hierarchical pore architecture makes these biogenic nanomembranes exceptionally mechanically stable, while maintaining a short pore length and a high porosity. The most prominent issue when replacing machined silica nanopore membranes with biogenic membranes is the initial random placement of the membranes on the solid substrate. This is also problematic when trying to accomplish a permanent fluidic seal around the membrane.

In our study, we demonstrate the ability to localize and immobilize a 200µm-diameter biomineralized nanopore membrane structure from marine algae, Coscinodiscus wailesii, on pre-defined positions on micro-machined silicon substrates. The substrates feature micron-sized through-wafer channels that allow easy access to the nanopore membrane. Localization of the membrane structure is accomplished using patterning of 8 µm thick hydrophobic resin. The addition of poly-L-lysine to the surface before solution-depositing the nanopore membranes results in a strong electrostatic binding force between the oxidized silicon platform and the diatom membranes. Lift-off of the photoresist in acetone removes randomly placed nanopore membranes on the resist-coated area, not affecting diatoms adhering to the silicon surface.

While poly-L-lysine provides an initial fluidic seal, permanent immobilization is accomplished by using UV-curable photoresist SU-8 and proximity photo lithography. Scanning electron micrographs after processing show intact diatoms without the presence of stress cracks. While initial electrochemical measurements indicate that some of the nanopores are clogged by residual epoxy resist after development, subsequent sulfuric-peroxide mixture (SPM) treatment removes the residual resist. Successful translocation experiments using polystyrene beads shows presence of unclogged pores, also indicating that the pore size of the biogenic silica nanomembranes can be modified by chemical treatment.

2680-Pos Board B699

Mixed Company in a Protein Nanopore: Transient Double Occupancy Enables Direct Exchange of Pore Ligand in Nanopore-Based Single Molecule Sensing

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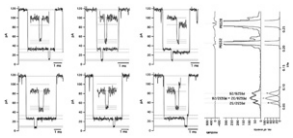
Partitioning of poly(ethylene-glycol) (PEG) into an alpha-hemolysin nanopore gives rise to long-lasting blockades ($\tau \approx 0.1$-10 ms) of ionic current. The depth of blockade shows exquisite sensitivity for polymer length, yielding mass spectra with single monomer resolution in the range between MW ≈ 700-2200 or 15-50 repeat units (r.u.). Unexpectedly, high-resolution recordings of single PEG blocking events using a mixture of two monodisperse species unequivocally identified direct transitions between levels corresponding to 28 and 32 r.u. without an intervening unblocked interval. Closer analysis revealed that these occur by the intermediary of shorter, deeper blocks. Based on statistics and current amplitude distributions, we are able to identify three such deeply blocked states, each corresponding to the simultaneous presence in the pore of two ligands: either 2xPEG-28, 2xPEG-32 or PEG-28+PEG-32 (mixed double occupancy). Direct PEG28->PEG32 transitions (or vice versa) are observed only with an intervening mixed occupancy block, which, however, can also result in return to the first blocked level. We conclude that the alpha-hemolysin pore is capable of accomodating two PEG oligomers, with ligand exchange occurring by displacement and translocation of the first blocker.



2681-Pos Board B700

Nanopores with Fluid Walls for Determining the Shape, Dipole Moment, and Rotational Diffusion Coefficient of Non-Spherical Proteins

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Recording ionic current through electrolyte-filled nanopores during the passage of proteins is an emerging technique for characterizing unmodified proteins in their native, aqueous environment. By measuring the reduction in current, ΔI, during the translocation of single proteins through an electrolyte-filled nanopore, this technique can characterize the size, charge, conformation, assembly and activity of hundreds of unlabeled proteins per second. For non-spherical proteins, however, broad distributions of ΔI values make estimates of protein size unreliable. This work employs lipid-bilayer coated nanopores and describes quantitative procedures for determining the shape and volume of single spherical and non-spherical proteins from distributions of ΔI values. Since the ΔI(t) signal is related to the orientation of non-spherical proteins in the nanopore, individual resistive-pulses can be used to determine the rotational diffusion coefficient and dipole moment of non-spherical proteins while in the nanopore. Moreover, this method has the potential to detect transient changes in the conformation of flexible proteins (e.g. an IgG antibody). This work extends the power of nanopores for characterizing proteins by adding the parameters of shape, volume, rotational diffusion coefficient, and dipole moment of non-spherical proteins to those that can already be determined in a single experiment such as the volume of spherical proteins, charge, and affinity for a ligand.

2682-Pos Board B701

Threading Immobilized DNA through a Solid-State Nanopore with a Tip

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Nanopores have been of interest for scientific research in addition to medical applications since they have the ability to detect and characterize single biomolecules with potentially high throughput and low cost. The Scanning Probe Microscope (SPM) method has sub-nanometer spatial resolution. We have constructed a combined SPM-solid state nanopore apparatus to study the capture and release process of lambda-DNA by a voltage biased solid-state nanopore. By tethering the DNA to an fiber tip in ionic solution, we can control the position of one end of the DNA molecule precisely, allowing us to study the DNA capture and release distance from the nanopore. We also have detected DNA sticking to the nanopore mouth without translocation through, it produced small current blockage, and we can study this process with one DNA molecule repetitively. This tethered DNA nanopore sensing method will provide a means to slow DNA translocation, allowing more detailed features of single DNA molecules to be studied, and potentially can be used with all types of nanopores with single-biomolecule sensitivity at controlled translocation rates.

2683-Pos Board B702

Brownian Dynamics Simulations of DNA Interaction with a Nanoporous Solid State Membrane

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We have developed computational model that allows us to study the influence of a nanoporous solid state membrane on the dynamics of a biomolecule. We apply various electrolyte and membrane biases and monitor the effects on DNA translocation and extension. The translocation of DNA through a nanopore in a single layered doped semiconductor membrane is studied. With our single layered, electrically tunable membranes, the DNA translocation time can be varied by more than one order of magnitude. Nanopore functionalization is also studied by fixing one strand of DNA to the inner surface of the pore. Two different models of DNA molecule are developed. The first model represents each DNA nucleotide as a single bead, while in the second one we consider two beads per nucleotide: one bead representing the phosphate and sugar backbone, and the other being the base. This model is more realistic and allows us to better understand the principles of interaction between the semiconductor membrane and DNA nucleotides.

2684-Pos Board B703

Ligand-Targeted Binding of a Novel Silicone Magnetic Microsphere

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In ligand-targeted drug delivery, a carrier particle conjugated with a ligand binds preferentially to an overexpressed receptor on the membrane of a specific cell type. A therapeutic agent is adsorbed onto or absorbed within the carrier, and its release is often triggered by magnetic stimulation or other means. In

this work, we demonstrate ligand-targeted delivery with a novel magnetic microsphere by conjugating the drug carriers with a folic acid ligand that preferentially binds to HeLa cells overexpressing folic acid receptors. The microspheres used in this study are produced in-house and contain magnetite nanoparticles (~10 nm) distributed uniformly throughout an amine-functionalized silicone matrix. The sphere diameter is scalable from 0.5 to 2.0 microns, and the concentration of magnetic nanoparticles can be varied up to 50% wt. The silicon matrix of this carrier facilitates compatibility with lipophilic drugs, the high magnetic content allows the potential for magnetically-stimulated drug release, and an abundance of primary amines within the matrix enables surface functionalization with a variety of ligands. Microspheres in this study were conjugated with folic acid using an EDAC reaction and tagged with a fluorophore. The spheres were incubated with HeLa cells, which overexpress folate-binding protein, and the degree of binding after 30 minutes was analyzed with fluorescence microscopy. We show a five-fold increase in bound spheres per cell relative to a control sphere without folic acid, indicating a high degree of specific binding. The preferential binding of ligand-conjugated magnetic microspheres gives insight into the utility of these drug carriers for targeted drug delivery studies.

2685-Pos Board B704

The Styrene-Maleic Acid Copolymer Extracts Active Complexes from Native Biomembranes

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Amphipathic polymers have been widely used to maintain the solubility of membrane proteins and complexes following detergent solubilization. However, their ability to extract proteins directly from lipid bilayers has remained largely unexplored. Here we show that a copolymer composed of styrene and maleic acid pendant groups (SMA) extracts proteins form native membranes and reconstitutes them into polymer-bound lipoprotein particles. First, we found that the SMA copolymer disrupted the membranes of intact mitochondria in a concentration-dependent and saturable manner. This was evidenced by the collapse of the transmembrane electric field of the inner mitochondrial membrane and by the solubilization of mitochondrial membrane proteins, both of which were mediated by the SMA copolymer in a manner similar to that mediated by nonionic detergents. Second, following incubation of the SMA copolymer with mitochondrial membranes and chromatographic separation, we observed by transmission electron microscopy that the resulting polymer-bound particles were a monodisperse population of discoids. The dimensions of these particles were similar to those previously reported for particles derived from liposomes or proteoliposomes of synthetic phospholipids (Lipodisqs®). Finally, using mitochondrial respiratory Complex IV (cytochrome c oxidase) as a model enzyme, we demonstrate that the SMA copolymer can extract even large, multicomponent complexes from native lipid bilayers and maintain them in a fully functional state amenable to solution-based biophysical studies. This novel approach to membrane protein reconstitution obviates the requirement for detergents and is therefore better suited to preserving native annular lipids and protein stability in comparison with traditional solubilization techniques.

2686-Pos Board B705

Hierarchical Accessibility of Double-Helical RNA and DNA Processing Signals in Highly Dense Self-Assemblies

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The ability to accurately detect biomolecules in single-cell amounts is an important goal of basic and translational research. The inherent capability of nucleic acids to self-assemble allows the spontaneous formation of highly dense, self-assembled monolayers (SAMs). These assemblages can provide the basis for development of nano-based devices with programmable, single-molecule detection capability. Double-stranded(ds) RNA is an important biomarker of viral infection and certain cancers. While dsRNA behavior in solution has been extensively characterized by diverse physicochemical approaches, the properties of highly dense assemblies of dsRNA are largely unknown, and may be qualitatively different from those in solution. High-density, 39 bp, chimeric dsRNA:dsDNA (ds-chimera) SAMs have been prepared on modified gold surfaces. using atomic force microscopy (AFM)-based nanomanipulation and analysis, and fluorescence microscopy, we demonstrate the preferential binding of ethidium to the ds-chimera SAM compared to the single-stranded form. As revealed by AFM detection of height change, the ds-chimera SAM is reactive towards the dsRNA-specific RNase III of Aquifex aeolicus (Aa-RNase III) and restriction endonuclease BamHI, each having a recognition site in the dsRNA and dsDNA segments, respectively. We also show that the reactivity of the BamHI cleavage site, which is proximal to the gold surface, can be controlled by SAM density, and that access to the BamHI site is dependent upon the prior action of RNase III at its site in the center of the ds-chimera. These results reveal novel properties of protein-nucleic acid interactions within a high-density array environment that are relevant to nanoscale detection methodologies.

2687-Pos Board B706

A Kinesin Driven Microfluidic Concentrator Device for Ultrasensitive Detection of Analyte

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The discovery of a vast array of biomarkers has spurred the demand for diagnostic assays with lower detection limits for early disease detection. Microfluidics makes it possible to work with small sample volumes and has played a significant role in creating more sensitive diagnostic tools. Our goal is to adapt our previous biomolecular motor (kinesin) based concentrator (*NanoLett.***8**:1041) and integrate antibody-functionalized microtubules into the device. Transforming this device into an immunoassay platform allows a variety of proteins or biomarkers to be actively captured and concentrated for detection. We believe this concentrator can improve typical ELISA assays by integrating two key features. First, concentrating the analyte-carrying microtubules into a small 625μm^2 concentrator region increases the signal to noise ratio allowing for more sensitive fluorescence measurements. Second, by ensuring that the binding capacity of these functionalized microtubules is high, we allow for a large number of antibodies and antigen to be concentrated. To achieve these goals we have developed a protocol to covalently link a high density of monoclonal, polyclonal or f(Ab)$_2$ antibodies onto microtubules without significantly affecting the motility of the complex. Motility is critical for the device since the microtubules with captured and fluorescently-labeled analyte are rapidly transported by kinesin into the concentrator region. The intensity of the resulting fluorescent signal in the concentrator region directly corresponds to the concentration of analyte in the initial sample. Our results show that the fluorescence intensity of individual anti-BSA coated microtubules allows the detection of sub-picomolar concentrations of TMR-BSA by integrating the fluorescence signals along microtubules. In conclusion, our data suggest that integrating functionalized microtubules and raising the signal to noise ratio by concentration in this device improves the detection limits of a typical ELISA assay while significantly reducing the assay time.

2688-Pos Board B707

Towards Nucleotide Differentiation with Solid-State Nanopores

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Nanopores have made significant progress toward a viable ''$1000 genome'' since their discovery just over a decade ago. To date, however, solid state nanopores have not demonstrated the resolution and signal power necessary to discriminate between different nucleotides or short polymer chains.

Here we report on the detection and discrimination of short chains of nucleotides. We use ultra-thin membranes(1), reproducibly fabricate sub-2nm nanopores, and exploit our proven ability to sample at high frequencies with low noise(2). Combining these experimental features enables measurements with high-sensitivity, high-signal, and low signal-to-noise, which allow us to detect different molecules through our nanopores.

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2. Rosenstein, J.K., Wanunu, M., Merchant, C.A., Drndic, M., and Shepard, K.L. 2012. Integrated nanopore sensing platform with sub-microsecond temporal resolution. Nature Methods 9:487-U112.

2689-Pos Board B708

A Binary Molecular Gate Made of DNA and Protein

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Many proteins have evolved the ability to recognize and bind to specific sequences of DNA. This specificity is quite high and often is the basis for genetic switches which can repress or induce transcription in cells. One of the best known DNA-binding proteins is the lac repressor protein which binds tightly to the lac operator but loses its affinity for this site upon the binding of lactose. We have used this lac repressor protein and the lac operator sequence to